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Ordering and lyotropic behavior of a silicon-supported cationic and neutral lipid system studied by neutron reflectivity

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Self-assembling of amphipathic lipid films on solid support allows the structural investigation of important biological model systems, such as the vectorlike lipid membranes, in order to improve DNA transfection in nonviral gene therapy. We present a neutron reflectivity study of a binary lipid system composed of dioleoylphosphatidylcholine (DOPC) and dimethyldioctadecylammonium bromide (DDAB) deposited on [100] silicon support by means of spin coating technique. We underline their lyotropic behavior under saturated deuterium oxide (D_2O) vapor thus pointing out that the lipid mixture is organized in ordered domains composed of plane lamellar bilayers of noninteractive DOPC and DDAB. © *2008 American Institute of Physics*.
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Monolayer and multilayer solid supported lipid nanofilms are considered as an attractive and useful model system for biological membranes[1,2] and have been extensively studied as a peculiar class of materials because of their potential applications in various fields.[3,4] Despite this, many structural features of such systems still have not been unraveled mainly due to their amphipathic behavior.

Many techniques, such as small angle x-ray scattering,[5] x-ray and neutron reflectivity,[6] atomic force microscopy,[7] and infrared near field microscopy,[8] have been employed to reveal the structural properties of these systems. In particular, by means of neutron reflectivity applied to the study of amphipathic systems, it is feasible to investigate the structure of solvated hydrophilic heads using deuterated water and also to evaluate the structural behavior of the aggregation with the same high sensitivity both on ionic or polar sector and on the hydrocarburic apolar one.[6,9] This paper aims at establishing the structural vertical organization of the thin film composed of amphiphilic molecules which are believed to play a pivotal role in nonviral gene therapy.[5,10] DNA transfection into cells can be effectively engineered through the use of cationic lipid/DNA "lipoplexes," although their transfection efficiency is sensitive to the neutral "helper" lipid included.[11,12] Morphologic features of each cationic lipid/helper system can be understood using solid supported membrane models in order to evaluate the actual role played by the helper in forming and maintaining lipid-DNA linkage.[11] Therefore, experiments were performed using neutron reflectivity technique[13] on the helper neutral crystal liquid dioleoylphosphatidylcholine (DOPC) on the cationic crystal gel dimethyldioctudecylammonium bromide (DDAB) and on the lipid mixture DOPC-DDAB, all spread by spin coating on silicon substrate chemically etched. It has been recently reported[14] that the spin-coating technique generates a homogeneous thin film, made by a limited and controlled number of lipid layers by varying the parameters of deposition (amount and concentration of lipid solution and rotational speed). Such systems are characterized by a low roughness,

thus, permitting the decrease in the perturbations induced by diffusive scattering on the exchanged momentum Q_z of neutrons reflected by the interfaces. In our previous paper,[6] we found that the mixture composed of cationic dioleoyltrimethyl-ammonium propane (DOTAP) and neutral DOPC lipids, which was deposited on silicon wafers by spin coating, was ordered as multiple bilayers with the presence of micron-sized clusters and DNA did not manage to organize itself within the mixture. For this reason, particular attention has been devoted to enlighten the noninteracting DOPC and DDAB sample contribution with respect to the properties shown by their mutual interaction. In order to compare the results obtained by neutron reflectivity under the same environmental conditions, the same samples have been studied through energy dispersion X-ray diffraction (EDXD) at grazing incident angle (0.45°) in Bragg geometry, using a chamber under controlled relative humidity and temperature.

DDAB (MW=631) and DOPC (MW=786.13) (mw denotes molecular weight) purchased from Sigma-Aldrich, (St. Louis Missouri, USA) were solved in chloroform at a 5 mg/ml concentration. In order to work in excess of helper conditions, but still with a DDAB amount so that a good reflectivity signal intensity can be obtained, we prepared the DOPC-DDAB chloroform solution, with a molecular weight fraction of Φ=DOPC/DOPC+DDAB=0.7. After a precleaning treatment using ethanol and trichloroethane, [100] silicon wafers were prepared by wet chemical etching to provide hydrogenated surfaces suitable for interaction with low dipolar electric moment solvent.[14] After 10 min of cleaning using a mixture of bidistillated (dd) water, hydrogen peroxide, and chloridric acid solution at 8:2:1 ratio, the wafers were immersed for 15 min in a 10% fluoridric acid solution. Finally, the obtained H-terminated supports were cleaned with dd H_2O and placed in a dry box to remove every trace of water and to prevent hydrogenated silicon to rapidly oxidize.[15] A constant amount of lipid solution was deposited on etched surface with a Convac spinner model 1001 (Convac Technologies, Sichuan China), selecting a sample spin speed of 3000 rpm. Neutron reflectivity experiments were performed at the CRISP energy dispersion reflectometer at

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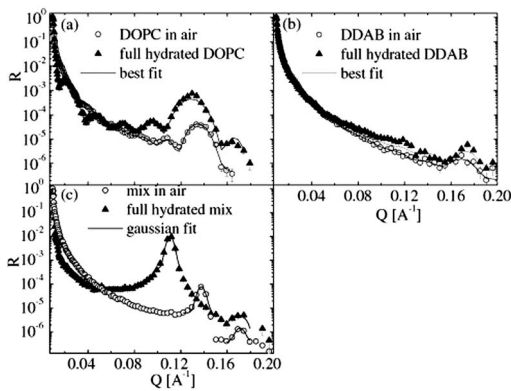
FIG. 1. Reflectivity spectra of (a) DOPC, (b) DDAB, and (c) DOPC/DDAB mixture (mix), in full hydrated and nonhydrated conditions.

the ISIS pulsed neutron source facility (Rutherford Appleton Laboratory). Starting from the reflectivity patterns, it is possible to calculate the scattering length density (SLD) profile thus mapping the density of the scatterers in the direction normal to the surface. This interfacial profile can be analyzed to determine a film's total thickness, material composition, periodicity, and roughness.[13] We performed the reflectivity measurements with nonhydrated samples in air conditioned at 20 °C and 50% relative humidity (RH) environment. CRISP facility is endowed with a hermetic hydration chamber by means of which the lyotropic response of water-exposed lipid multilayers can be observed. In order to maximize the SLD contrast, the samples were hydrated in saturated D_2O vapor conditions (RH=100%). D_2O was permeated within the cell, without wetting the sample[16] and the chamber was carefully kept at 20 °C. The equilibrium between the chemical potential of D_2O saturated vapor and that of D_2O absorbed from the lipid film was assessed by means of low-statistic preliminary measurements, within regular amounts of time.

The results of individual DOPC and DDAB on silicon wafer obtained on full hydrated condition (RH=100%) were compared with those obtained in air and shown in Figs. 1(a) and 1(b) respectively, for the two lipids. Neutron reflectivity measurements, obtained at 0.25°, 0.6° and 1.4°, were combined together in order to investigate a broad Q_Z range. DOPC deposited on silicon sample, after being prepared and assessed twice, allowed us to verify the measurement reproducibility. A model SLD profile was generated starting from the physical characteristics of the system, and used to calculate the reflectivity curve, through the iterative dynamic method.[17] The physical model we chose depicts a homogeneous lipid film as mainly built up by a periodic assembly of a double layer of heads alternated with a double layer of tails. If necessary, further independent layers adjacent to the periodic multilayer, generally as film edges, were inserted. The SLD profile as a function of the film height, starting from the surface layer and moving toward the substrate was obtained by least-squares iterative procedure. Data analysis was performed using PARRATT32 software.[18] The individual lipids in 50% RH conditions, appear vertically located in a plane lamellar structure composed of six double layers plus an independent external layer. The main morphological information we obtained are summarized in Table I. D_2O presence into lipid films determines an enhanced SLD contrast allowing to increase the quality of the measurement and to gain some information about the completely hydrated structure behavior. In Fig. 2, we show the comparison between

TABLE I. Physical parameters of full hydrated and unhydrated single lipids.

Physical parameters	DOPC [a]In air	Full hydrated	DDAB [a]In air	Full hydrated
Bilayers number In air Full hydrated	[a]6 bilayers+1 distinct layer	5 bilayers+1 distinct layer	[a]6 bilayers+1 distinct layer	7 bilayers
Heads SLD (Å⁻²)	[a]1.36×10^{-6}	2.92×10^{-6}	[a]1.18×10^{-6}	4.02×10^{-7}
Heads roughness (Å)	[a]8	5	[a]9	1
Tails SLD (Å⁻²)	[a]-1.28×10^{-7}	-4.34×10^{-7}	[a]-3.68×10^{-7}	-2.40×10^{-7}
Tails roughness (Å)	[a]7	2	[a]9	6
Head vertical extension (Å)	[a]7.7 ± 0.1	10.9 ± 0.1	[a]3.2 ± 0.1	3.8 ± 0.1
Head spherical section (Å²)	[a]46 ± 1	93 ± 1	[a]9 ± 1	11 ± 1
Tail vertical extension (Å)	[a]15.1 ± 0.1	13.2 ± 0.1	[a]14.6 ± 0.1	13.3 ± 0.1
Tail cylinder section (Å³)	[a]860 ± 1	270 ± 1	[a]490 ± 1	750 ± 1
Lattice constant (Å)	[a]45.6 ± 0.4	48.2 ± 0.4	[a]35.6 ± 0.4	34.3 ± 0.4
Independent layer SLD (Å⁻²)	[a]2.64×10^{-7}	2.61×10^{-7}		
vertical extension (Å)	[a]32 ± 1	71 ± 1		
Film thickness (Å)	[a]353 ± 1	390 ± 1	[a]240 ± 1	240 ± 1

the DOPC and DDAB SLD profiles both in air and in full hydration conditions, evidencing that the layer adjacent to the substrate is made up of hydrated heads. Moreover, we assessed that whereas a lyotropic mesophase is associated with the DOPC hydrated structure, causing a polar head section swelling[19,20] and a simultaneous thinning of hydrocarbon portion, D_2O presence upon DDAB determines only a little increase in the head section coupled to a light bilayers compression. D_2O molecules adsorption increases the volume of each phosphatidylcholine of DOPC determining an enhancement of the reticular constant found in Fig. 1(a). From such swelling, we estimate an increase of a 15 water molecules per lipid which relates to a 50% RH increase.[21] Both the loss of the ordering in normal direction (*z*) of a double layer and the increase of the thickness of the external independent layer of the hydrated DOPC film (Table I), leads us to the hypothesis of a detachment of the more external bilayer. In fact, superficial instabilities are due to the 100% RH condition.[22] The little increase of hydrated DOPC lattice
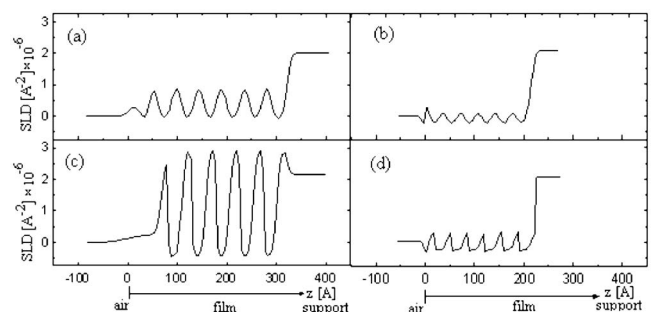

FIG. 2. SLD profiles of (a) nonhydrated DOPC, (b) nonhydrated DDAB, (c) full hydrated DOPC, and (d) full hydrated DDAB.
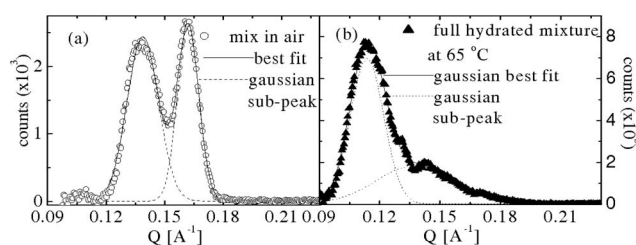
FIG. 3. EDXD spectra of the biphasic mixture thin film (a) in air, with two Bragg peaks and (b) after 1 h at 65 °C in full hydrated condition.

constant and the thickness of the choline head layer adjacent to the substrate help the ordered film observed in hydrated DOPC to increase in thickness. Conversely to hydrated DOPC, measurements performed on DDAB sample by means of EDXD (data not shown), confirm the weak tendency of this system to let water enter into multilayers and the stability of the film under a relative humidity gradient. This behavior is due both to the peculiar DDAB molecular structure (i.e., the presence of Br^- and the absence of hydrophilic linker between the hydrophobic and the hydrophilic part) and also to its aggregation state as a crystalline gel.[23–26] In particular, the Br^- presence affects DDA^+ phase behavior, as demonstrated in Ref. 19, attenuating the repulsive electric field amidst the heads, promoting the hydrocarbon chains interactions and conferring the structure a low value of hydrophilic-lipophilic balance. Such assessment could explain the observed low interacting affinity between the two mixture components.[27,28] In fact, the reflectivity curve obtained on the mixture in air condition and reported in Fig. 1(c) clearly shows the presence of two Bragg peaks centered at 0.138 and 0.17 $Å^{-1}$, corresponding to the lamellar spacing of the single lipids DOPC and DDAB, respectively (see Table I). Such coincidence proves that every single component of the mixture forms a separate lamellar structure with the same lattice constant of the single lipids [Figs. 1(a) and 1(b)]. Moreover, Fig. 1(c) shows the behavior of the mixture exposed to saturated D_2O vapor underlining again two lamellar phases. The first one presumably consists of DOPC domains with a notable increase of the lattice constant ($d = 56.7$ Å) in the hydrated mixture, whereas the second one, characterized by a diffraction peak at 0.17 $Å^{-1}$, corresponds to hydrated DDAB sample profile. Lattice constant increase, due to deuterated water adsorbed among the heads of each bilayer, happens to be higher in DOPC domains present in the mixture with respect to the pure sample (see Table I). In fact, due to a rather disorganized bordered interfacial layer among the domains of heterogeneous lipid structures, an increase in water and small ions diffusion capacity has been noticed.[29,30] The mixed system has not been modeled and only a Gaussian model of the Bragg peaks zone of the reflectivity profile was generated. Thus, in order to further validate such results, we performed EDXD measurements on the same DOPC-DDAB mixture [Fig. 3(a)]. Such measurements, performed in different regions of the sample, confirm that the mixture is organized in distinct vertical ordered domains of not mixed DOPC and DDAB. In order to stress the influence of the physical aggregation state of DDAB on such cluster formation, in Fig. 3(b), we show the EDXD spectrum of the solid supported DOPC-DDAB mixture treated in water-saturated vapor environment at a temperature higher with respect to that of crystal gel—crystal

liquid DDAB phase transition.[26] Temperature induces the dissolution of such clusters on behalf of a single crystal-liquid mesophase which we associated to the higher Bragg peak (54.8 Å); however, the geometrical ordering is not yet clear (work in progress). Such thermal-lyotropic transition, in fact, does not appear to be complete throughout all the points of the support surface and the other two small peaks shown in Fig. 3(b), centered at Q_z= 0.143 $Å^{-1}$ and 0.168 $Å^{-1}$, could be related to the presence of the individual not dissolved lipids. The different lyotropic behavior of single lipids leads us to make the hypothesis that DDAB would be clusterized in DOPC also in liposomes composed of these two molecules, dispersed in water solution at room temperature, weakening the helper role in favoring the lipid/DNA complex formation. For this reason, the same samples prepared with different preparation methods will be analyzed also by other spectroscopic techniques to highlight that the properties of the aggregations are peculiar of the lipid components and not affected by the experimental method used.

[1] M. Tanaka and E. Sackmann, Nature (London) **437**, 656 (2005).
[2] R. P. Richter, R. Bérat, and A. R. Brisson, Langmuir **22**, 3497 (2006).
[3] E. Sackmann, Science **271**, 43 (1996).
[4] F. Davis and S. P. J. Higson, Biosens. Bioelectron. **21**, 1 (2005).
[5] I. Koltover, T. Salditt, J. O. Rädler, and C. R. Safinya, Science **78**, 281 (1998).
[6] J. Generosi, C. Castellano, D. Pozzi, and A. Congiu Castellano, J. Appl. Phys. **96**, 6839 (2004).
[7] G. Pompeo, M. Girasole, A. Cricenti, F. Cattaruzza, A. Flamini, T. Prosperi, J. Generosi, and A. Congiu Castellano, Biochim. Biophys. Acta **1712**, 29 (2005).
[8] J. Generosi, G. Margaritondo, J. S. Sanghera, I. D. Aggarwal, N. H. Tolk, D. W. Piston, A. Congiu Castellano, and A. Cricenti, Appl. Phys. Lett. **89**, 233906 (2006).
[9] T. Gutberlet, R. Steitz, J. Howse, I. Estrela-Lopis, and B. Klösgen, Appl. Phys. A: Mater. Sci. Process. **74**, s1262 (2002).
[10] L. Wasungu and D. Hoekstra, J. Controlled Release **116**, 255 (2006).
[11] S. W. Hui, M. Langner, Y. L. Zhao, P. Ross, E. Hurley, and K. Chan, Biophys. J. **71**, 590 (1996).
[12] P. Callow, G. Fragneto, R. Cubitt, D. J. Barlow, M. J. Lawrence, and P. Timmins, Langmuir **21**, 7912 (2005).
[13] J. Penfold and R. K. Thomas, J. Phys.: Condens. Matter **2**, 1369 (1990).
[14] U. Mennicke and T. Salditt, Langmuir **18**, 8172 (2002).
[15] M. Maccarini, M. Himmelhaus, S. Stoycheva, and M. Gruze, Appl. Surf. Sci. **252**, 1941 1946 (2005).
[16] L. Perino-Gallice, G. Fragneto, U. Mennicke, T. Salditt, and F. Rieutord, Eur. Phys. J. E **8**, 275 (2002).
[17] L. G. Parratt, Phys. Rev. **95**, 359 (1954).
[18] C. Braun, The reflectivity tool, PARRATT32 version 1.5.2, (Hahn-Meitner Institute, Berlin, 1999).
[19] M. Ge and J. H. Freed, Biophys. J. **85**, 4023 (2003).
[20] R. J. Mashl, H. L. Scott, S. Subramaniam, and E. Jakobsson, Biophys. J. **81**, 3005 (2001).
[21] H. I. Petrache, S. E. Feller, and J. F. Nagle, Biophys. J. **72**, 2237 (1997).
[22] T. Salditt, C. Li, A. Spaar, and U. Mennicke, Eur. Phys. J. E **7**, 105 (2002).
[23] A. Devinat (private communication).
[24] F. Ricoul, M. Dubois, T. Zemp, A. Vandais, J.-P. Noel, M. Lefevre, D. Plusquellec, and O. Diat, Prog. Colloid Polym. Sci. **105**, 351 (1997).
[25] E. Feitosa, P. C. A. Barreleiro, and G. Olofsson, Chem. Phys. Lipids **105**, 201 (2000).
[26] E. Feitosa, F. R. Alves, A. Niemiec, E. C. D. R. Oliveira, E. M. S. Castanheira, and A. L. Baptista, Langmuir **22**, 3579 (2006).
[27] L. J. Johnston, Langmuir **23**, 5886 (2007).
[28] R. Elliott, K. Katsov, M. Schick, and I. Szleifer, J. Chem. Phys. **122**, 044904 (2005).
[29] C. Nicolini and A. Rigo, *Biofisica e Tecnologie Biomediche* (Zanichelli, Bologna, 1996), pp. 302–304.
[30] L. Cruzeiro-Hansson, J. H. Ipsen, and O. G. Mouritsen, Biochim. Biophys. Acta **27**, 166 (1989).